



**Patent Office
Canberra**

I, MARIO PERUSSICH, ASSISTANT DIRECTOR PATENT SERVICES, hereby certify that the annexed are true copies of the Provisional specification and drawing(s) as filed on 1 November 1996 in connection with Application No. PO 3384 for a patent by THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH filed on 1 November 1996.

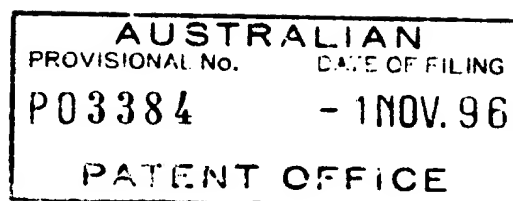
I further certify that the annexed documents are not, as yet, open to public inspection.

WITNESS my hand this Fourth
day of October 1997

1

A handwritten signature in black ink, appearing to read "Mario Perussich", written over a horizontal line.

MARIO PERUSSICH
ASSISTANT DIRECTOR PATENT SERVICES



THE WALTER AND ELIZA HALL
INSTITUTE OF MEDICAL RESEARCH

A U S T R A L I A
Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"THERAPEUTIC AND DIAGNOSTIC AGENTS"

The invention is described in the following statement:

- 1A-

THERAPEUTIC AND DIAGNOSTIC AGENTS

The present invention relates generally to therapeutic and diagnostic agents. More particularly, the present invention provides therapeutic molecules capable of modulating
5 signal transduction such as but not limited to cytokine-mediated signal transduction. The molecules of the present invention are useful, therefore, in modulating cellular responsiveness to cytokines as well as cytokine-mediated immune response mechanisms.

Bibliographic details of the publications referred to in this specification by author are collected
10 at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be
15 understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Cytokines are secreted proteins that regulate the survival, proliferation, differentiation and function of a variety of cells within the body [Nicola, 1994]. The haemopoietic cytokines
20 have in common a four-alpha helical bundle structure and the vast majority interact with a structurally related family of cell surface receptors, the type I and type II cytokine receptors [Bazan, 1990; Sprang, 1993]. In all cases ligand-induced receptor aggregation appears to be a critical event in initiating intracellular signal transduction cascades. Some cytokines, for example growth hormone, erythropoietin (Epo) and granulocyte colony stimulating factor,
25 trigger receptor homodimerisation, while for other cytokines, receptor heterodimerisation or heterotrimerisation is crucial. In the latter cases, several cytokines share common receptor subunits and on this basis can be grouped into three subfamilies with similar patterns of intracellular activation and similar biological effects [Hilton, 1994]. Interleukin-3 (IL-3), IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF) use the common β -
30 receptor subunit (β c), and each cytokine stimulates the production and functional activity of

- 2 -

granulocytes and macrophages. IL-2, IL-4, IL-7, IL-9, and IL-15 each use the common γ -chain (γ_c), while IL-4 and IL-13 share an alternative γ -chain (γ'_c or IL-13 receptor α -chain). Each of these cytokines plays an important role in regulating acquired immunity in the lymphoid system. Finally, IL-6, IL-11, leukaemia inhibitory factor (LIF), oncostatin-M (OSM), ciliary neurotrophic factor (CNTF) and cardiotrophin (CT) share the receptor subunit gp130. Each of these cytokines appears to be highly pleiotropic, having effects both within and outside the haemopoietic system [Nicola, 1994].

In all of the above cases at least one subunit of each receptor complex contains the conserved sequence elements, termed box1 and box2, in their cytoplasmic tails [Murakami, 1991]. Box1 is a proline-rich motif which is located more proximal to the transmembrane domain than the acidic box 2 element. The box-1 region serves as the binding site for a class of cytoplasmic tyrosine kinases termed JAKs (Janus kinases). Ligand-induced receptor dimerisation serves to increase the catalytic activity of the associated JAKs through cross-phosphorylation. Activated JAKs then tyrosine phosphorylate several substrates, including the receptors themselves. Specific phosphotyrosine residues on the receptor then serve as docking sites for SH2-containing proteins, the best characterised of which are the signal transducers and activators of transcription (STATs) and the adaptor protein, shc. The STATs are then phosphorylated on tyrosines, probably by JAKs, dissociate from the receptor and form either homodimers or heterodimers through the interaction of the SH2 domain of one STAT with the phosphotyrosine residue of the other. STAT dimers then translocate to the nucleus where they bind to specific cytokine-responsive promoters and activate transcription [Darnell, 1994; Ihle, 1995; Ihle, 1995]. In a separate pathway, tyrosine phosphorylated shc interacts with another SH2 domain-containing protein, Grb-2, leading ultimately to activation of members of the MAP kinase family and in turn transcription factors such as fos and jun [Sato, 1993; Cutler, 1993]. These pathways are not unique to members of the cytokine receptor family, with cytokines that bind receptor tyrosine kinases also being able to activate STATs and members of the MAP kinase family [David, 1996; Leaman, 1996; Shual, 1993; Sato, 1993; Cutler, 1993].

- 3 -

Four members of the JAK family of cytoplasmic tyrosine kinases have been described, JAK1, JAK2, JAK3 and TYK2, each of which binds to a specific subset of cytokine receptor subunits. Six STATs have been described (STAT1 through STAT6), and these too are activated by distinct cytokine/receptor complexes. For example, STAT1 appears to be functionally specific to the interferon system, STAT4 appears to be specific to IL-12, while STAT6 appears to be specific for IL-4 and IL-13. Thus, despite common activation mechanisms some degree of cytokine specificity may be achieved through the use of specific JAKs and STATs [Thierfelder, 1996; Kaplan, 1996; Kaplan, 1996; Takeda, 1996; Shimoda, 1996; Meraz, 1996; Durbin, 1996].

10

In addition to those described above, there are clearly other mechanisms of activation of these pathways. For example, the JAK/STAT pathway appears to be able to activate MAP kinases independent of the shc-induced pathway [David, 1995] and the STATs themselves can be activated without binding to the receptor, possibly by direct interaction with JAKs [Gupta, 1996]. Conversely, full activation of STATS may require the action of MAP kinase in addition to that of JAKs [David, 1995; Wen, 1995].

15

While the activation of these signalling pathways is becoming better understood, little is known of the negative feedback loops.

20

In work leading up to the present invention, the inventors sought to isolate negative regulators of signal transduction. The inventors have now identified a new family of SH2 domain-containing proteins which are capable of acting as regulators of cytokine signalling. The identification of this new family of cytokine regulatory molecules permits the generation of a range of molecules capable of modulating cytokine signal transduction. The SOCS define a new family of negative regulators of signal transduction.

25

The regulatory molecules of the present invention are referred to as suppressors of cytokine signalling or "SOCS". Specific SOCS are defined numerically, for example, SOCS1, SOCS2 and SOCS3.

30

- 4 -

Accordingly, one aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an SH2 domain-containing protein or a derivative thereof which protein suppresses cytokine signal
5 transduction.

More particularly, the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an SH2 domain-containing protein or a derivative thereof which protein suppresses cytokine signal
10 transduction mediated by at least one of IL-6, LIF, OSM, IFN- γ or thrombopoietin.

Even more particularly, the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an SH2 domain-containing protein or a derivative thereof which protein suppresses cytokine
15 signal transduction mediated by IL-6 in M1 cells expressing said nucleic acid molecule.

Another aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an SH2 domain-containing protein or a derivative thereof which protein modulates cellular responsiveness to
20 cytokines.

More particularly, the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an SH2 domain-containing protein or a derivative thereof which protein modulates cellular responsiveness to
25 one or more of IL-6, LIF, OSM, IFN- γ or thrombopoietin.

Even more particularly, the present invention relates to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an SH2 domain-containing protein or a derivative thereof which protein modulates cellular
30 responsiveness to IL-6 when tested in M1 cells expressing said nucleic acid molecule.

- 5 -

The present invention further contemplates an isolated protein or a derivative thereof comprising a sequence of amino acids having an SH2 domain and which is capable of regulating cytokine signal transduction.

- 5 Another aspect provides an isolated protein or a derivative thereof comprising a sequence of amino acids having an SH2 domain and which is capable of regulating cytokine signal transduction for one or more of IL-6, LIF, OSM, IFN- γ or thrombopoietin.

Still another aspect of the present invention is directed to an isolated protein or a derivative
10 thereof comprising a sequence of amino acids having an SH2 domain and which is capable of regulating cytokine signal transduction mediated by IL-6 in M1 cells expressing said protein.

Yet still another aspect of the present invention provides an isolated protein or a derivative
15 thereof comprising a sequence of amino acids having an SH2 domain and which is capable of modulating cellular responsiveness to cytokines.

In a related embodiment, the present invention is directed to an isolated protein or a derivative thereof comprising a sequence of amino acids having an SH2 domain and which is capable
20 of modulating cellular responsiveness to one or more of IL-6, LIF, OSM, IFN- γ or thrombopoietin.

A further embodiment provides an isolated protein or a derivative thereof comprising a sequence of amino acids having an SH2 domain and which is capable of modulating cellular
25 responsiveness to IL-6 when tested in M1 cells expressing said protein.

In accordance with these aspects of the present invention, the nucleic acid molecules encode a SOCS or a derivative thereof. Reference to a "SOCS" includes reference to family members of SOCS such as SOCS1, SOCS2 and SOCS3. SOCS have an SH2 domain. They
30 also comprise a C-terminal domain referred to as a C-terminal SOCS domain. Preferably,

- 6 -

the SOCS is in recombinant form although the present invention extends to a naturally occurring SOCS in isolated or purified form. The terms "isolated" and "purified" means that a molecule has undergone at least one purification step away from other material.

5 Preferably, the nucleic acid molecule is in isolated form and is DNA such as cDNA or genomic DNA. The DNA may encode the same amino acid sequence as in the naturally occurring SOCS or the SOCS may contain one or more amino acid substitutions, deletions and/or additions. The nucleotide sequence may correspond to the genomic coding sequence or to the nucleotide sequence in cDNA of the naturally occurring mRNA or may carry one
10 or more nucleotide substitutions, deletions and/or additions thereto.

In a preferred embodiment, the nucleotide sequence of the SOCS, regardless of from which sub-family, comprises the sequence set forth in SEQ ID NO:3 or has at least 15 % homology thereto and/or is capable of hybridising the nucleotide sequence set forth in SEQ ID NO:3
15 under low stringency conditions. The nucleotide sequence set forth in SEQ ID NO:3 encodes SOCS1. Within a sub-family, i.e. intra-sub-family, the level of homology is higher, such as at least about 40 %.

Accordingly, the present invention contemplates a nucleotide sequence encoding a particular
20 SOCS, and preferably SOCS1 comprising a sequence set forth in SEQ ID NO:3 or having at least 40 % homology thereto and/or is capable of hybridising to the nucleotide sequence of SEQ ID NO:3 under low stringency conditions.

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1%
25 v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at
30 least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which

- 7 -

includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions.

- 5 In a related embodiment, the nucleotide sequence encodes an amino acid sequence as set forth in SEQ ID NO:4 (referred to herein as SOCS1) or has at least 15 % similarity thereto.

The above sequence comparisons are preferably to the whole molecule but may also be to part thereof.

10

Preferred nucleotide percentage similarities include at least about 20 %, at least about 40 %, at least about 50 %, at least about 60 %, at least about 70 %, at least about 80 %, at least about 90 % or above such as 93 %, 95 %, 98 % or 99 %.

- 15 Preferred amino acid similarities include at least about 20 %, at least about 30 %, at least about 40 %, at least about 50 %, at least about 60 %, at least about 70 %, at least about 80 %, at least about 90 %, at least about 95 %, at least about 97 % or 98 % or above.

The nucleic acid molecule may be isolated from any animal such as humans, livestock animals
20 (e.g. horses, cows, sheep, donkeys, pigs), laboratory test animals (e.g. mice, rats, rabbits, hamsters, guinea pigs or primates), companion animals (e.g. dogs, cats) or captive wild animals (e.g. deer, foxes, kangaroos, monkeys and other primates).

The terms "derivatives" or its singular form "derivative" whether in relation to a nucleic acid
25 molecule or a protein includes parts, mutants, fragments and analogues as well as hybrid or fusion molecules and glycosylation variants. Preferably, the derivatives have functional activity or alternatively act as antagonists or agonists.

- 8 -

One example of an antagonist is an antisense oligonucleotide sequence. Useful oligonucleotides are those which have a nucleotide sequence complementary to at least a portion of the protein-coding or "sense" sequence of the nucleotide sequence. These antisense nucleotides can be used to effect the specific inhibition of gene expression. The
5 antisense approach can cause inhibition of gene expression apparently by forming an antiparallel duplex by complementary base pairing between the antisense construct and the targeted mRNA, presumably resulting in hybridisation arrest of translation. Ribozymes and co-suppression molecules may also be used. Antibodies may also act as either antagonists or agonists.

10

The present invention extends to analogues of the SOCS proteins of the present invention and their use in the treatment or prophylaxis of cytokine mediated dysfunction such as autoimmunity, immune suppression or hyperactive immunity. Analogues of the proteins contemplated herein include, but are not limited to, modification to side chains, incorporating
15 of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecule or their analogues.

Examples of side chain modifications contemplated by the present invention include
20 modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with NaBH_4 ; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-
25 phosphate followed by reduction with NaBH_4 .

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

30

- 9 -

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

10

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

15

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acid, contemplated herein is shown in Table 1.

25

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with $n=1$ to $n=6$, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH).

30

In addition, peptides can be conformationally constrained by, for example, incorporation of C α and N α -methylamino acids, introduction of double bonds between C α and C β atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

These types of modifications may be important to stabilise the cytokines if administered to an individual or for use as a diagnostic reagent.

10

TABLE 1

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α -aminobutyric acid	Abu	L-N-methylalanine	Nmala
α -amino- α -methylbutyrate	Mgab	L-N-methylarginine	Nmarg
aminocyclopropane- carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl- carboxylate	Norb	L-N-methylglutamine	Nmgln
		L-N-methylglutamic acid	Nmglu
cyclohexylalanine		Chexa L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn

- 11 -

	D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
	D-isoleucine	Dile	L-N-methylproline	Nmpro
	D-leucine	Dleu	L-N-methylserine	Nmser
	D-lysine	Dlys	L-N-methylthreonine	Nmthr
5	D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
	D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
	D-phenylalanine	Dphe	L-N-methylvaline	Nmval
	D-proline	Dpro	L-N-methylethylglycine	Nmetg
	D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
10	D-threonine	Dthr	L-norleucine	Nle
	D-tryptophan	Dtrp	L-norvaline	Nva
	D-tyrosine	Dtyr	α -methyl-aminoisobutyrate	Maib
	D-valine	Dval	α -methyl- γ -aminobutyrate	Mgab
	D- α -methylalanine	Dmala	α -methylcyclohexylalanine	Mchexa
15	D- α -methylarginine	Dmarg	α -methylcyclopentylalanine	Mcpen
	D- α -methylasparagine	Dmasn	α -methyl- α -naphthylalanine	Manap
	D- α -methylaspartate	Dmasp	α -methylpenicillamine	Mpen
	D- α -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D- α -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
20	D- α -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
	D- α -methylisoleucine	Dmile	N-amino- α -methylbutyrate	Nmaabu
	D- α -methylleucine	Dmleu	α -naphthylalanine	Anap
	D- α -methyllysine	Dmlys	N-benzylglycine	Nphe
	D- α -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
25	D- α -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
	D- α -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D- α -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D- α -methylserine	Dmser	N-cyclobutylglycine	Ncbut
	D- α -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
30	D- α -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex

- 12 -

	D- α -methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
	D- α -methylvaline	Dmval	N-cylcododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
5	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
	D-N-methylglutamine	Dnmglu	N-(3-guanidinopropyl)glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
10	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
	D-N-methylleucine	Dnmleu	N-(3-indolylethyl)glycine	Nhtrp
	D-N-methyllysine	Dnmlys	N-methyl- γ -aminobutyrate	Nmgabu
	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmt
15	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpn
	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
20	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
	D-N-methyltyrosine	Dnmtyr	N-methyl-naphthylalanine	Nmanap
	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	γ -aminobutyric acid	Gabu	N-(<i>p</i> -hydroxyphenyl)glycine	Nhtyr
	L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
25	L-ethylglycine	Etg	penicillamine	Pen
	L-homophenylalanine	Hphe	L- α -methylalanine	Mala
	L- α -methylarginine	Marg	L- α -methylasparagine	Masn
	L- α -methylaspartate	Masp	L- α -methyl- <i>t</i> -butylglycine	Mtbug
	L- α -methylcysteine	Mcys	L-methylethylglycine	Metg
30	L- α -methylglutamine	Mglu	L- α -methylglutamate	Mglu

- 13 -

L- α -methylhistidine	Mhis	L- α -methylhomophenylalanine	Mhphe
L- α -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
L- α -methylleucine	Mleu	L- α -methyllysine	Mlys
L- α -methylmethionine	Mmet	L- α -methylnorleucine	Mnle
5 L- α -methylnorvaline	Mnva	L- α -methylornithine	Morn
L- α -methylphenylalanine	Mphe	L- α -methylproline	Mpro
L- α -methylserine	Mser	L- α -methylthreonine	Mthr
L- α -methyltryptophan	Mtrp	L- α -methyltyrosine	Mtyr
L- α -methylvaline	Mval	L-N-methylhomophenylalanine	Nmhph
10 N-(N-(2,2-diphenylethyl)	Nnbhm	N-(N-(3,3-diphenylpropyl)	Nnbhe
carbamylmethyl)glycine		carbamylmethyl)glycine	
1-carboxy-1-(2,2-diphenyl-	Nmbc		
ethylamino)cyclopropane			

15

Other derivatives contemplated by the present invention include a range of glycosylation variants from a completely unglycosylated molecule to a modified glycosylated molecule. Altered glycosylation patterns may result from expression of recombinant molecules in different host cells.

20

Another embodiment of the present invention contemplates a method for modulating expression of a SOCS protein in a mammal, said method comprising contacting a gene encoding a SOCS or a factor/element involved in controlling expression of the SOCS gene with an effective amount of a modulator of SOCS expression for a time and under conditions

25 sufficient to up-regulate or down-regulate or otherwise modulate expression of SOCS. An example of a modulator is a cytokine such as IL-6 or other transcription regulators of SOCS expression.

Expression includes transcription or translation or both.

30

- 14 -

Another aspect of the present invention contemplates a method of modulating activity of SOCS in a human, said method comprising administering to said mammal a modulating effective amount of a molecule for a time and under conditions sufficient to increase or decrease SOCS activity. The molecule may be a proteinaceous molecule or a chemical entity
5 and may also be a derivative of SOCS or a chemical analogue or truncation mutant of SOCS.

A further aspect of the present invention provides a method of inducing synthesis of a SOCS or transcription/translation of a SOCS comprising contacting a cell containing a SOCS gene with an effective amount of a cytokine capable of inducing said SOCS for a time and under
10 conditions sufficient for said SOCS to be produced. For example, SOCS1 may be induced by IL-6.

Accordingly, the present invention contemplates a pharmaceutical composition comprising SOCS or a derivative thereof or a modulator of SOCS expression or SOCS activity and one
15 or more pharmaceutically acceptable carriers and/or diluents. These components are referred to as the "active ingredients".

Although these and other aspects of the present invention apply to any SOCS, one preferred SOCS is SOCS1. Other preferred SOCS molecules include SOCS2 and SOCS3.
20

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion or may be in the form of a cream or other form suitable for topical application. It must be stable under the conditions of manufacture and storage and
25 must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required
30 particle size in the case of dispersion and by the use of surfactants. The preventions of the

- 15 -

action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the
5 compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by
10 incorporating the various sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously
15 sterile-filtered solution thereof.

When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be
20 incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may
25 conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 μ g and 2000 mg of active compound.

- 16 -

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter: A binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such a sucrose, lactose or
5 saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound,
10 sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and formulations.

15

The present invention also extends to forms suitable for topical application such as creams, lotions and gels.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion
20 media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

25

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the
30 desired therapeutic effect in association with the required pharmaceutical carrier. The

- 17 -

specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which
5 bodily health is impaired as herein disclosed in detail.

The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active
10 compound in amounts ranging from 0.5 μ g to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 μ g to about 2000 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

15 The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule capable of modulating SOCS expression or SOCS activity. The vector may, for example, be a viral vector.

20 Still another aspect of the present invention is directed to antibodies to SOCS and its derivatives. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to SOCS or may be specifically raised to SOCS or derivatives thereof. In the case of the latter, SOCS or its derivatives may first need to be associated with
25 a carrier molecule. The antibodies and/or recombinant SOCS or its derivatives of the present invention are particularly useful as therapeutic or diagnostic agents.

For example, SOCS and its derivatives can be used to screen for naturally occurring antibodies to SOCS. These may occur, for example in some autoimmune diseases. Alternatively,
30 specific antibodies can be used to screen for SOCS. Techniques for such assays are well

- 18 -

known in the art and include, for example, sandwich assays and ELISA. Knowledge of SOCS levels may be important for diagnosis of certain cancers or a predisposition to cancers or monitoring cytokine mediated cellular responsiveness or for monitoring certain therapeutic protocols.

5

Antibodies to SOCS of the present invention may be monoclonal or polyclonal. Alternatively, fragments of antibodies may be used such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic antibody" is considered herein to include fragments and hybrids of antibodies.

10 The antibodies of this aspect of the present invention are particularly useful for immunotherapy and may also be used as a diagnostic tool for assessing apoptosis or monitoring the program of a therapeutic regimen.

For example, specific antibodies can be used to screen for SOCS proteins. The latter would
15 be important, for example, as a means for screening for levels of SOCS in a cell extract or other biological fluid or purifying SOCS made by recombinant means from culture supernatant fluid. Techniques for the assays contemplated herein are known in the art and include, for example, sandwich assays and ELISA.

20 It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies or synthetic antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region
25 of SOCS.

Both polyclonal and monoclonal antibodies are obtainable by immunization with the enzyme or protein and either type is utilizable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily
30 prepared by injection of a suitable laboratory animal with an effective amount of SOCS, or

antigenic parts thereof, collecting serum from the animal, and isolating specific sera by any of the known immunoabsorbent techniques. Although antibodies produced by this method are utilizable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

5

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be
10 done by techniques which are well known to those who are skilled in the art.

Another aspect of the present invention contemplates a method for detecting SOCS in a biological sample from a subject said method comprising contacting said biological sample with an antibody specific for SOCS or its derivatives or homologues for a time and under
15 conditions sufficient for an antibody-SOCS complex to form and then detecting said complex.

The presence of SOCS may be accomplished in a number of ways such as by Western blotting and ELISA procedures. A wide range of immunoassay techniques are available as can be seen by reference to US Patent Nos. 4,016,043, 4,424,279 and 4,018,653. These, of course,
20 include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target.

Sandwich assays are among the most useful and commonly used assays and are favoured for
25 use in the present invention. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilized on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second
30 antibody specific to the antigen, labelled with a reporter molecule capable of producing a

- 20 -

detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In accordance with the present invention the sample is one which might contain SOCS including cell extract, tissue biopsy or possibly serum, saliva, mucosal secretions, lymph, tissue fluid and respiratory fluid. The sample is, therefore, generally a biological sample comprising biological fluid but also extends to fermentation fluid and supernatant fluid such as from a cell culture.

In the typical forward sandwich assay, a first antibody having specificity for the SOCS or antigenic parts thereof, is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes) and under suitable conditions (e.g. 25°C) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the hapten. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the hapten.

- 21 -

An alternative method involves immobilizing the target molecules in the biological sample and then exposing the immobilized target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with the antibody.

- 5 Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its
10 chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody,
15 generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the
20 corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate
25 substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which was present in the sample. "Reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state to excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

The present invention also contemplates genetic assays such as involving PCR analysis to detect SOCS gene or its derivatives. Alternative methods or methods used in conjunction include direct nucleotide sequencing or mutation scanning such as single stranded conformation polymorphisms analysis (SSCP) as specific oligonucleotide hybridisation, as methods such as direct protein truncation tests.

The nucleic acid molecules of the present invention may be DNA or RNA. When the nucleic acid molecule is in DNA form, it may be genomic DNA or cDNA. RNA forms of the nucleic acid molecules of the present invention are generally mRNA.

Although the nucleic acid molecules of the present invention are generally in isolated form, they may be integrated into or ligated to or otherwise fused or associated with other genetic molecules such as vector molecules and in particular expression vector molecules. Vectors and expression vectors are generally capable of replication and, if applicable, expression in one or both of a prokaryotic cell or a eukaryotic cell. Preferably, prokaryotic cells include *E. coli*, *Bacillus sp* and *Pseudomonas sp*. Preferred eukaryotic cells include yeast, fungal, mammalian and insect cells.

Accordingly, another aspect of the present invention contemplates a genetic construct comprising a vector portion and a mammalian and more particularly a human SOCS gene portion, which SOCS gene portion is capable of encoding a SOCS polypeptide or a functional or immunologically interactive derivative thereof.

5

Preferably, the SOCS gene portion of the genetic construct is operably linked to a promoter on the vector such that said promoter is capable of directing expression of said SOCS gene portion in an appropriate cell.

- 10 In addition, the SOCS gene portion of the genetic construct may comprise all or part of the gene fused to another genetic sequence such as a nucleotide sequence encoding glutathione-S-transferase or part thereof.

The present invention extends to such genetic constructs and to prokaryotic or eukaryotic cells
15 comprising same.

The present invention also extends to any or all derivatives of SOCS including mutants, part, fragments, portions, homologues and analogues or their encoding genetic sequence including single or multiple nucleotide or amino acid substitutions, additions and/or deletions to the
20 naturally occurring nucleotide or amino acid sequence.

The SOCS and its genetic sequence of the present invention will be useful in the generation of a range of therapeutic and diagnostic reagents and will be especially useful in the detection of a cytokine involved in a particular cellular response or a receptor for that cytokine. For
25 example, cells expressing SOCS gene such as M1 cells expressing the SOCS1 gene, will no longer be responsive to a particular cytokine such as in the case of SOCS1, IL-6.

Soluble SOCS polypeptides are also contemplated to be particularly useful in the treatment of disease, injury or abnormality involving cytokine mediated cellular responsiveness such as
30 hyperimmunity, immunosuppression, allergies, hypertension and the like.

- 24 -

A further aspect of the present invention contemplates the use of SOCS or its functional derivatives in the manufacture of a medicament for the treatment of conditions involving cytokine mediated cellular responsiveness.

5

The present invention further contemplates transgenic mammalian cells expressing a SOCS gene. Such cells are useful indicator cell lines for assaying for suppression of cytokine function. One example is M1 cells expressing the SOCS1 gene. Such cell lines may be useful for screening for cytokines or screening molecules such as naturally occurring molecules from
10 plants, coral, microorganisms or bio-organically active soil or water capable of acting as cytokine antagonists or agonists.

The present invention is further described by the following non-limiting Figures and Examples.

15

In the Figures:

Figure 1 is a diagrammatic representation showing generation of an IL-6-unresponsive M1 clone by retroviral infection. The RUFneo retrovirus, showing the position of landmark
20 restriction endonuclease cleavage sites, the 4A2 cDNA insert and the position of PCR primer sequences.

Figure 2 is a photographic representation of Southern and Northern analysis. (Left and Middle Panels) Southern blot analysis of genomic DNA from clone 4A2 and a control
25 infected M1 clone. DNA was digested with BamH I, to reveal the number of retroviruses carried by each clone, and Sac I, to estimate the size of the retroviral cDNA insert. Left panel; probed with neo. Right panel; probed with the Xho I-digested 4A2 PCR product. (Right Panel) . Northern blot analysis of total RNA from clone 4A2 and a control infected M1 clone, probed with the Xho I-digested 4A2 PCR product. The two bands represent
30 unspliced and spliced retroviral transcripts, resulting from splice donor and acceptor sites

in the retroviral genome.

Figure 3 is a representation of the nucleotide sequence and structure of the SOCS1 gene. A. The genomic context of SOCS1 in relation to the protamine gene cluster on murine chromosome 16. The accession number of this locus is MPMRNGNS (direct submission; G. Schlueter, 1995) for the mouse and BTPRMTNP2 for the rat (direct submission; G. Schlueter, 1996). B. The nucleotide sequence of the SOCS1 cDNA and deduced amino acid sequence. Conventional one letter abbreviations are used for the amino acid sequence and the asterisk indicates the stop codon. The polyadenylation signal sequence is underlined. The coding region is shown in uppercase and the untranslated region is shown in lower case.

Figure 4 is a graphical representation of cell differentiation in the presence of cytokines. Semi-solid agar cultures of parental M1 cells (M1 and M1.mpl) and M1 cells expressing SOCS1 (4A2 and M1.mpl.SOCS1), were used and the percentage of colonies which differentiated in response to a titration of 1 mg/ml IL-6 (●), 100 ng/ml LIF (◆), 1 mg/ml OSM (■), 100 ng/ml IFN-γ (▲), 500 ng/ml TPO (●), or 3x10⁻⁶ M dexamethasone (*) determined.

Figure 5 is a photographic representation of cytopins of liquid cultures of parental M1 cells (M1 and M1.mpl)) and M1 cells expressing SOCS1 (4A2 and M1.mpl.SOCS1) cultured for 4 days in the presence of 10 ng/ml IL-6 or saline. Unlike parental M1 cells, morphological features consistent with macrophage differentiation are not observed in M1 cells constitutively expressing SOCS1 (4A2 and M1.mpl.SOCS1) when cultured in IL-6.

Figure 6 is a photographic representation showing inhibition of phosphorylation of signalling molecules by SOCS-1. Parental M1 cells (M1 and M1.mpl) and M1 cells expressing SOCS-1 (4A2 and M1.mpl.SOCS-1) were incubated in the absence (-) or presence (+) of 10 ng/ml of IL-6 for 4 minutes at 37°C . Cells were then lysed and extracts were either immunoprecipitated using anti-mouse gp130 antibody prior to SDS-PAGE (two upper panels) or were electrophoresed directly (two lower panels). Gels were blotted and the filters

were then probed with anti-phosphotyrosine (upper panel), anti-gp130 antibody (second top panel), anti-phospho-STAT3 (second bottom panel) or anti-STAT3 (lower panel). Blots were visualised using peroxidase-conjugated secondary antibodies and Enhanced Chemiluminescence (ECL) reagents.

5

Figure 7 is a representation of protein extracts prepared from (A) M1 cells or M1 cells expressing SOCS-1 (4A2) and (B) M1.mpl cells or M1.mpl.SOCS-1 cells incubated for 10 min at 37°C in 10 ml serum-free DME containing either saline, 100 ng/ml IL-6 or 100 ng/ml IFN- γ . The binding reactions contained 4-6 μ g protein (constant within a given
10 experiment), 5 ng 32 P-labelled m67 oligonucleotide encoding the high affinity SIF (c-sis-inducible factor) binding site, and 800 ng sonicated salmon sperm DNA. For certain experiments, protein samples were preincubated with an excess of unlabelled m67 oligonucleotide, or antibodies specific for either STAT1 or STAT3.

15 **Figure 8** is a photographic representation of Northern hybridisation. Mice were injected intravenously with 2 μ g and after various periods of time, the livers were removed and polyA+ mRNA was purified. M1 cells were stimulated for various lengths of time with 500 ng/ml of IL-6, after which polyA+ mRNA was isolated. mRNA was fractionated by electrophoresis and immobilized on nylon filters. Northern blots were prehybridised,
20 hybridized with random-primed 32 P-labelled SOCS-1 or GAPDH DNA fragments, washed and exposed to film overnight.

- 27 -

EXAMPLE 1

CELL CULTURE AND CYTOKINES

The M1 cell line was derived from a spontaneously arising leukaemia in SL mice [Ichikawa, 1969]. Parental M1 cells used in this study have been in passage at the Walter and Eliza
5 Hall Institute for Medical Research, Melbourne, Victoria, Australia, for approximately 10 years. M1 cells were maintained by weekly passage in Dulbecco's modified Eagle's medium (DME) containing 10% (v/v) foetal bovine serum (FCS). Recombinant cytokines are generally available from commercial sources or were prepared by published methods. Recombinant murine LIF was produced in *Escherichia coli* and purified, as previously
10 described [Gearing, 1989]. Purified human oncostatin M was purchased from PeproTech Inc (Rocky Hill, NJ, USA), and purified mouse IFN- γ was obtained from Genzyme Diagnostics (Cambridge, MA, USA). Recombinant murine thrombopoietin was produced as a FLAGTM-tagged fusion protein in CHO cells and then purified.

15

EXAMPLE 2

AGAR COLONY ASSAYS

In order to assay the differentiation of M1 cells in response to cytokines, 300 cells were cultured in 35 mm Petri dishes containing 1 ml of DME supplemented with 20% (v/v) fetal calf serum (FCS), 0.3% (w/v) agar and 0.1 ml of serial dilutions of IL-6, LIF, OSM, IFN- γ ,
20 tpo or dexamethasone (Sigma Chemical Company, St Louis, MI). After 7 days culture at 37°C in a fully humidified atmosphere, containing 10% (v/v) CO₂ in air, colonies of M1 cells were counted and classified as differentiated if they were composed of dispersed cells or had a corona of dispersed cells around a tightly packed centre.

25

EXAMPLE 3

GENERATION OF RETROVIRAL LIBRARY

A cDNA expression library was constructed from the factor-dependent haemopoietic cell line FDC-P1, essentially as described [Rayner, 1994]. Briefly, cDNA was cloned into the retroviral vector pRUFneo and then transfected into an amphotrophic packaging cell line
30 (PA317). Transiently generated virus was harvested from the cell supernatant at 48 hr

- 28 -

posttransfection, and used to infect Y2 ecotropic packaging cells, to generate a high titre virus-producing cell line.

EXAMPLE 4

5 RETROVIRAL INFECTION OF M1 CELLS

Pools of 10^6 infected Ψ 2 cells were irradiated (3000 rad) and cocultivated with 10^6 M1 cells in DME supplemented with 10%(v/v) FCS and 4 μ g/ml Polybrene, for 2 days at 37°C. To select for IL-6-unresponsive clones, retrovirally-infected M1 cells were washed once in DME, and cultured at approximately 2×10^4 cells/ml in 1 ml agar cultures containing 400
10 μ g/ml geneticin (GibcoBRL, Grand Island, NY) and 100 ng/ml IL-6. The efficiency of infection of M1 cells was 1-2%, as estimated by agar plating the infected cells in the presence of geneticin only.

EXAMPLE 5

15 PCR

Genomic DNA from retrovirally-infected M1 cells was digested with Sac I and 1 μ g of phenol/chloroform extracted DNA was then amplified by polymerase chain reaction (PCR). Primers used for amplification of cDNA inserts from the integrated retrovirus were GAG3 (5' CACGCCGCCCCACGTGAAGGC 3' [SEQ ID NO:1]), which corresponds to the vector
20 gag sequence approximately 30 bp 5' of the multiple cloning site, and HSVTK (5' TTCGCCAATGACAAGACGCT 3' [SEQ ID NO:2]), which corresponds to the pMC1neo sequence approximately 200 bp 3' of the multiple cloning site. The PCR entailed an initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 2 min, and extension at 72°C for 3 min, followed by a final 10 min extension.
25 PCR products were gel purified and then ligated into the pGEM-T plasmid (Promega, Madison, WI), and sequenced using an ABI PRISM Dye Terminator Cycle Sequencing Kit and a Model 373 Automated DNA Sequencer (Applied Biosystems Inc., Foster City, CA).

EXAMPLE 6

SOUTHERN AND NORTHERN BLOT ANALYSES

- ³²P-labelled probes were generated using a random decanucleotide labelling kit (Bresatec, Adelaide, South Australia) from a 600 bp Pst I fragment encoding neomycin phosphotransferase from the plasmid pPGKneo, 1070 bp fragment of the SOCS1 gene obtained by digestion of the 1.4 kbp PCR product with Xho I, SOCS2, SOCS3, CIS and a 1.2 kbp fragment of the chicken glyceraldehyde 3-phosphate dehydrogenase gene [Dugaiczky, 1983].
- 10 Genomic DNA was isolated from cells using a proteinase K-sodium dodecyl sulfate procedure essentially as described. Fifteen micrograms of DNA was digested with either BamH I or Sac I, fractionated on a 0.8%(w/v) agarose gel, transferred to GeneScreenPlus membrane (Du Pont NEN, Boston MA), prehybridised, hybridised with random-primed ³²P-labelled DNA fragments and washed essentially as described [Sambrook, 1989].
- 15 Total RNA was isolated from cells and tissues using Trizol Reagent, as recommended by the manufacturer (GibcoBRL, Grand Island, NY). When required polyA⁺ mRNA was purified essentially as described [Alexander, 1995]. Northern blots were prehybridised, hybridized with random-primed ³²P-labelled DNA fragments and washed as described [Alexander,
- 20 1995].

EXAMPLE 7

DNA CONSTRUCTS AND TRANSFECTION

- A cDNA encoding epitope-tagged SOCS1 was generated by subcloning the entire SOCS1 coding region into the pEF-BOS expression vector [Mizushima, 1990], engineered to encode an inframe FLAG epitope downstream of an initiation methionine (pF-SOCS1). Using electroporation as described previously [Hilton, 1994], M1 cells expressing the thrombopoietin receptor (M1.mpl) were transfected with the 20 µg of Aat II-digested pF-SOCS1 expression plasmid and 2 µg of a Sca I-digested plasmid in which transcription of
- 25 a "cDNA" encoding puromycin N-acetyl transferase was driven from the mouse
- 30

- 30 -

phosphoglycerokinase promoter (pPGK_{luc}proA). After 48 hours in culture, transfected cells were selected with 20 μ g/ml puromycin (Sigma Chemical Company, St Louis MO), and screened for expression of SOCS1 by Western blotting, using the M2 anti-FLAG monoclonal antibody according to the manufacturer's instructions (Eastman Kodak, 5 Rochester NY). In other experiments M1 cells were transfected with only the pF-SOCS1 plasmid or a control and selected by their ability to grow in agar in the presence of 100 ng/ml of IL-6.

EXAMPLE 8

10 IMMUNOPRECIPITATION AND WESTERN BLOTTING

Prior to either immunoprecipitation or Western blotting, 10⁷ M1 cells or their derivatives were washed twice, resuspended in 1ml of DME, and incubated at 37°C for 30 min. The cells were then stimulated for 4 min at 37°C with either saline or 100 ng/ml IL-6, after which sodium vanadate (Sigma Chemical Co., St Louis, MI) was added to a concentration of 1 15 mM. Cells were placed on ice, washed once with saline containing 1 mM sodium vanadate, and then solubilised for 5 min on ice with 300 μ l 1% (v/v) Triton X-100, 150 mM NaCl, 2 mM EDTA, 50 mM Tris-HCl pH 7.4, containing Complete protease inhibitors (Boehringer Mannheim, Mannheim, Germany) and 1 mM sodium vanadate. Lysates were cleared by centrifugation and quantitated using a Coomassie Protein Assay Reagent (Pierce, 20 Rockford IL).

For immunoprecipitations, equal concentrations of protein extracts (1-2 mg) were incubated for 1 hr or overnight at 4°C with either 4 μ g of anti-gp130 antibody (M20; Santa Cruz Biotechnology Inc., Santa Cruz, CA) or 4 μ g of anti-phosphotyrosine antibody (4G10; 25 Upstate Biotechnology Inc., Lake Placid NY), and 15 μ l packed volume of Protein G Sepharose (Pharmacia, Uppsala, Sweden). Immunoprecipitates were washed twice in 1% (v/v) NP40, 150 mM NaCl, 50 mM Tris-HCl pH 8.0, containing Complete protease inhibitors (Boehringer Mannheim, Mannheim, Germany) and 1 mM sodium vanadate. The samples were heated for 5 min at 95°C in SDS sample buffer (625 mM Tris-HCl pH 6.8, 30 0.05% (w/v) SDS, 0.1% (v/v) glycerol, bromophenol blue, 0.125% (v/v) 2-

- 31 -

mercaptoethanol), fractionated by SDS-PAGE and immunoblotted as described above.

For Western blotting, 10 μ g of protein from a cellular extract or material from an immunoprecipitation reaction was loaded onto 4-15% Ready gels (Bio-Rad Laboratories, Hercules CA), and resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred to PVDF membrane (Micron Separations Inc., Westborough MA) for 1 hr at 100 V. The membranes were probed with the following primary antibodies; anti-tyrosine phosphorylated STAT3 (1:1000 dilution; New England Biolabs, Beverly, MA); anti-STAT3 (C-20; 1:100 dilution; Santa Cruz Biotechnology Inc., Santa Cruz CA); anti-gp130 (M20, 1:100 dilution; Santa Cruz Biotechnology Inc., Santa Cruz CA); anti-phosphotyrosine (horseradish peroxidase-conjugated RC20, 1:5000 dilution; Transduction Laboratories, Lexington KY); anti-tyrosine phosphorylated MAP kinase and anti-MAP kinase antibodies (1:1000 dilution; New England Biolabs, Beverly, MA). Blots were visualised using peroxidase-conjugated secondary antibodies and Enhanced Chemiluminescence (ECL) reagents according to the manufacturer's instructions (Pierce, Rockford IL).

EXAMPLE 9

ELECTROPHORETIC MOBILITY SHIFT ASSAYS

Assays were performed as described [Novak, 1995], using the high affinity SIF (c-sis-inducible factor) binding site m67 [Wakao, 1994]. Protein extracts were prepared from M1 cells incubated for 10 min at 37°C in 10 ml serum-free DME containing either saline, 100 ng/ml IL-6 or 100 ng/ml IFN- γ . The binding reactions contained 4-6 μ g protein (constant within a given experiment), 5 ng 32 P-labelled m67 oligonucleotide, and 800 ng sonicated salmon sperm DNA. For certain experiments, protein samples were preincubated with an excess of unlabelled m67 oligonucleotide, or antibodies specific for either STAT1 (Transduction Laboratories, Lexington, KY) or STAT3 (Santa Cruz Biotechnology Inc., Santa Cruz CA), as described [Novak, 1995].

EXAMPLE 10

EXPRESSION CLONING OF A NOVEL SUPPRESSOR OF CYTOKINE SIGNAL TRANSDUCTION

In order to identify cDNAs capable of suppressing cytokine signal transduction, an expression cloning approach was adopted. This strategy centred on M1 cells, a monocytic leukaemia cell line that differentiates into mature macrophages and ceases proliferation in response to the cytokines IL-6, LIF, OSM and IFN- γ , and the steroid dexamethasone. Parental M1 cells were infected with the RUFneo retrovirus, into which cDNAs from the factor-dependent haemopoietic cell line FDC-P1 had been cloned. In this retrovirus, transcription of both the neomycin resistance gene and the cloned cDNA was driven off the powerful constitutive promoter present in the retroviral LTR (Figure 1). When cultured in semi-solid agar, parental M1 cells form large tightly packed colonies. Upon stimulation with IL-6, M1 cells undergo rapid differentiation, resulting in the formation in agar of only single macrophages or small dispersed clusters of cells. Retrovirally-infected M1 cells that were unresponsive to IL-6 were selected in semi-solid agar culture by their ability to form large, tightly packed colonies in the presence of IL-6 and geneticin. A single stable IL-6-unresponsive clone, 4A2, was obtained after examining 10^4 infected cells.

A fragment of the neomycin phosphotransferase (neo) gene was used to probe a Southern blot of genomic DNA from clone 4A2 and this revealed that the cell line was infected with a single retrovirus containing a cDNA approximately 1.4 kbp in length (Figure 2). PCR amplification using primers from the retroviral vector which flanked the cDNA cloning site enabled recovery of a 1.4 kbp cDNA insert, which we have named suppressor of cytokine signalling-1, or SOCS1. This PCR product was used to probe a similar Southern blot of 4A2 genomic DNA and hybridised to two fragments, one which corresponded to the endogenous SOCS1 gene and the other, which matched the size of the band seen using the neo probe, corresponded to the SOCS1 cDNA cloned into the integrated retrovirus (Figure 2). The latter was not observed in an M1 cell clone infected with a retrovirus containing an irrelevant cDNA. Similarly, Northern blot analysis revealed that SOCS1 mRNA was abundant in the cell line 4A2, but not in the control infected M1 cell clone (Figure 2).

EXAMPLE 11
SOCS1, SOCS2, SOCS3 AND CIS DEFINE A NEW FAMILY
OF SH2-CONTAINING PROTEINS

The SOCS1 PCR product was used as a probe to isolate homologous cDNAs from a mouse
5 thymus cDNA library. The sequence of the cDNAs proved to be identical to the PCR
product, suggesting that constitutive or over expression, rather than mutation, of the SOCS1
protein was sufficient for generating an IL-6-unresponsive phenotype. Comparison of the
sequence of SOCS1 cDNA with nucleotide sequence databases revealed that it was present
on mouse and rat genomic DNA clones containing the protamine gene cluster found on
10 mouse chromosome 16. Closer inspection revealed that the 1.4 kb SOCS1 sequence was not
homologous to any of the protamine genes, but rather represented a previously unidentified
open reading frame located at the extreme 3' end of these clones (Figure 3). There were no
regions of discontinuity between the sequences of the SOCS1 cDNA and genomic locus,
suggesting that SOCS1 is encoded by a single exon. In addition to the genomic clone
15 containing the protamine genes, a series of murine and human expressed sequenced tags
(ESTs) also revealed large blocks of nucleotide sequence identity to mouse SOCS1. The
sequence information provided by the human ESTs allowed the rapid cloning of cDNAs
encoding human SOCS1.

20 The SOCS1 gene encoded a 212 amino acid protein. A search of translated nucleic acid
databases with the predicted amino acid sequence of SOCS1 showed that it was most related
to a recently cloned cytokine-inducible immediate early gene product, CIS, and two classes
of ESTs. Full length cDNAs from the two classes of ESTs were isolated and found to
encode proteins of similar length and overall structure to SOCS1 and CIS. These clones
25 were given the names SOCS2 and SOCS3. Each of the four proteins contains a central SH2
domain and a C-terminal region termed the SOCS motif. The SOCS1 proteins exhibit an
extremely high level of amino acid sequence similarity (95-98% identity) amongst different
species. However, the forms of the SOCS1, SOCS2, SOCS3 and CIS from the same
animal, while clearly defining a new family of SH2-containing proteins, exhibited a lower
30 amino acid identity.

- 34 -

EXAMPLE 12
CONSTITUTIVE EXPRESSION OF SOCS1 SUPPRESSES THE
ACTION OF A RANGE OF CYTOKINES

To formally establish that the phenotype of the 4A2 cell line was directly related to expression of SOCS1, and not to unrelated genetic changes which may have occurred independently in these cells, a cDNA encoding an epitope-tagged version of SOCS1 under the control of the EF1 α promoter was transfected into parental M1 cells, and M1 cells expressing the receptor for thrombopoietin, c-mpl (M1.mpl). Transfection of the SOCS1 expression vector into both cell lines resulted in an increase in the frequency of IL-6 unresponsive M1 cells.

The effect of constitutive SOCS1 expression on the response of M1 cells to a range of cytokines was investigated using the 4A2 cell line and a clone of M1.mpl cells expressing SOCS1 (M1.mpl.SOCS1). Unlike parental M1 cells and M1.mpl cells, the two cell lines expressing SOCS1 continued to proliferate and failed to form differentiated colonies in response to either IL-6, LIF, OSM, IFN- γ or, in the case of the M1.mpl.SOCS1 cell line, thrombopoietin (Figure 4). For both cell lines, however, a normal response to dexamethasone was observed, suggesting that SOCS1 specifically affected cytokine signal transduction rather than differentiation *per se*. Consistent with these data, while parental M1 cells and M1.mpl cells became large and vacuolated in response to IL-6, 4A2 and M1.mpl.SOCS1 cells showed no evidence of morphological differentiation in response to IL-6 or other cytokines (Figure 5).

- 35 -

EXAMPLE 13
SOCS1 INHIBITS A RANGE OF IL-6 SIGNAL TRANSDUCTION
PROCESSES, INCLUDING STAT3 PHOSPHORYLATION
AND ACTIVATION

5 Phosphorylation of the cell surface receptor component gp130, the cytoplasmic tyrosine kinase JAK1 and the transcription factor STAT3 is thought to play a central role in IL-6 signal transduction. These events were compared in the parental M1 and M1.mpl cell lines and their SOCS1-expressing counterparts. As expected, gp130 was phosphorylated rapidly in response to IL-6 in both parental lines, however, this was reduced five- to ten-fold in the
10 cell lines expressing SOCS1 (Figure 6). Likewise, STAT3 phosphorylation was also reduced by approximately ten-fold in response to IL-6 in those cell lines expressing SOCS1 (Figure 6). Consistent with a reduction in STAT3 phosphorylation, activation of specific STAT DNA binding complexes, as determined by electrophoretic mobility shift assay, was also reduced. Notably, there was a reduction in the formation of SIF-A (containing
15 STAT3), SIF-B (STAT1/STAT3 heterodimer) and SIF-C (containing STAT1), the three STAT complexes induced in M1 cells stimulated with IL-6 (Figure 7). Similarly, constitutive expression of SOCS1 also inhibited IFN- γ -stimulated formation of p91 homodimers (Figure 7). STAT phosphorylation and activation were not the only cytoplasmic processes to be effected by SOCS1 expression, as the phosphorylation of other
20 proteins, including shc and MAP kinase, was reduced to a similar extent (Figure 7).

EXAMPLE 14
TRANSCRIPTION OF THE SOCS1 GENE IS STIMULATED BY IL-6
IN VITRO AND IN VIVO

25 Although SOCS1 can inhibit cytokine signal transduction when constitutively expressed in M1 cells, this does not necessarily indicate that SOCS1 normally functions to negatively regulate an IL-6 response. In order to investigate this possibility the inventors determined whether transcription of the SOCS1 gene is regulated in the response of M1 cells to IL-6 and, because of the critical role IL-6 plays in regulating the acute phase response to injury
30 and infection, the response of the liver to intravenous injection of 5 mg IL-6. In the absence

- 36 -

of IL-6, SOCS1 mRNA was undetectable in either M1 cells or in the liver. However, for both cell types, a 1.4 kb SOCS1 transcript was induced within 20 to 40 minutes by IL-6 (Figure 8). For M1 cells, where the IL-6 was present throughout the experiment, the level of SOCS1 mRNA remained elevated (Figure 8). In contrast, IL-6 was administered in vivo
5 by a single intravenous injection and was rapidly cleared from the circulation, resulting in a pulse of IL-6 stimulation to the liver. Consistent with this, transient expression of SOCS1 mRNA was detectable in the liver, peaking approximately 40 minutes after injection and declining to basal levels within 4 hours (Figure 8).

10 Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more
15 of said steps or features.

BIBLIOGRAPHY:

Alexander WS, Metcalf D and Dunn AR (1995). Point mutations within a dimer interface homology domain of c-Mpl induce constitutive receptor activity and tumorigenicity. *Embo Journal* 14, 5569-78.

Bazan JF (1990). Haemopoietic receptors and helical cytokines. [Review]. *Immunology Today* 11, 350-4.

Cutler RL, Liu L, Damen JE and Krystal G (1993). Multiple cytokines induce the tyrosine phosphorylation of Shc and its association with Grb2 in hemopoietic cells. *Journal of Biological Chemistry* 268, 21463-5.

Darnell J Jr., Kerr IM and Stark GR (1994). Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. [Review]. *Science* 264, 1415-21.

David M, Petricoin E3, Benjamin C, Pine R, Weber MJ and Larner AC (1995). Requirement for MAP kinase (ERK2) activity in interferon alpha- and interferon beta-stimulated gene expression through STAT proteins [see comments]. *Science* 269, 1721-3.

David M, Wong L, Flavell R, Thompson SA, Wells A, Larner AC and Johnson GR (1996). STAT activation by epidermal growth factor (EGF) and amphiregulin. Requirement for the EGF receptor kinase but not for tyrosine phosphorylation sites or JAK1. *Journal of Biological Chemistry* 271, 9185-8.

Dugaiczky A, Haron JA, Stone EM, Dennison OE, Rothblum KN and Schwartz RJ (1983). Cloning and sequencing of a deoxyribonucleic acid copy of glyceraldehyde-3-phosphate dehydrogenase messenger ribonucleic acid isolated from chicken muscle. *Biochemistry* 22, 1605-13.

Durbin JE, Hackenmiller R, Simon MC and Levy DE (1996). Targeted disruption of the mouse Stat1 gene results in compromised innate immunity to viral disease. *Cell* 84, 443-50.

Gearing DP, Nicola NA, Metcalf D, Foote S, Willson TA, Gough NM and Williams L (1989). Production of leukaemia inhibitory factor in *Escherichia coli* by a novel procedure and its use in maintaining embryonic stem cells in culture. *BioTechnology* 7, 1157-1161.

Gupta S, Yan H, Wong LH, Ralph S, Krolewski J and Schindler C (1996). The SH2 domains of Stat1 and Stat2 mediate multiple interactions in the transduction of IFN- α signals. *Embo Journal* 15, 1075-84.

Hilton DJ (1994). An introduction to cytokine receptors, p8-16 *in* Guidebook to Cytokines and Their Receptors, Eds: N. A. Nicola. Oxford University Press: Oxford.

Hilton DJ, Hilton AA, Raicevic A, Rakar S, Harrison-Smith M, Gough NM, Begley CG, Metcalf D, Nicola NA and Willson TA (1994). Cloning of a murine IL-11 receptor α -chain; requirement for gp130 for high affinity binding and signal transduction. *Embo Journal* 13, 4765-75.

Ichikawa Y (1969). Differentiation of a cell line of myeloid leukemia. *Journal of Cellular Physiology* 74, 223-34.

Ihle JN (1995). Cytokine receptor signalling. [Review]. *Nature* 377, 591-4.

Ihle JN, Witthuhn BA, Quelle FW, Yamamoto K and Silvennoinen O (1995). Signaling through the hematopoietic cytokine receptors. [Review]. *Annual Review of Immunology* 13, 369-98.

Kaplan MH, Schindler U, Smiley ST and Grusby MJ (1996a). Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. *Immunity* 4, 313-9.

Kaplan MH, Sun YL, Hoey T and Grusby MJ (1996b). Impaired IL-12 responses and enhanced development of TH2 cells in STAT4 deficient mice. *Nature* 382, 174-179.

Leaman DW, Pisharody S, Flickinger TW, Commane MA, Schlessinger J, Kerr IM,

Levy DE and Stark GR (1996). Roles of JAKs in activation of STATs and stimulation of c-fos gene expression by epidermal growth factor. *Molecular & Cellular Biology* 16, 369-75.

Meraz MA, White JM, Sheehan KC, Bach EA, Rodig SJ, Dighe AS, Kaplan DH, Riley JK, Greenlund AC, Campbell D, Carver-Moore K, DuBois RN, Clark R, Aguet M and Schreiber RD (1996). Targeted disruption of the Stat1 gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway. *Cell* 84, 431-42.

Mizushima S and Nagata S (1990). pEF-BOS, a powerful mammalian expression vector. *Nucleic Acids Research* 18, 5322.

Murakami M, Narazaki M, Hibi M, Yawata H, Yasukawa K, Hamaguchi M, Taga T and Kishimoto T (1991). Critical cytoplasmic region of the interleukin-6 signal transducer gp130 is conserved in the cytokine receptor family. *Proc. Natl. Acad. Sci. USA* 88, 11349-11353.

Nicola NA((1994). *Guidebook to Cytokines and Their Receptors*. Oxford University Press: Oxford.

Novak U, Harpur AG, Paradiso L, Kanagasundaram V, Jaworowski A, Wilks AF and Hamilton JA (1995). Colony-stimulating factor 1-induced STAT1 and STAT3 activation is accompanied by phosphorylation of Tyk2 in macrophages and Tyk2 and JAK1 in fibroblasts. *Blood* 86, 2948-56.

Rayner JR and Gonda TJ (1994). A simple and efficient procedure for generating stable expression libraries by cDNA cloning in a retroviral vector. *Molecular & Cellular Biology* 14, 880-7.

Sambrook J, Fritsch EF and Maniatis T (1989). *Molecular Cloning, A Laboratory Manual*. Cold Spring Harbour Laboratory Press: Plainview.

Sato N, Sakamaki K, Terada N, Arai K and Miyajima A (1993). Signal transduction by the high-affinity GM-CSF receptor: two distinct cytoplasmic regions of the common beta subunit responsible for different signaling. *Embo Journal* 12, 4181-9.

Shimoda K, van Deursen J, Sangster MY, Sarawar SR, Carson RT, Tripp RA, Chu C, Quelle FW, Nosaka T, Vignali DA, Doherty PC, Grosveld G, Paul WE and Ihle JN (1996). Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. *Nature* 380, 630-3.

Shual K, Ziemiecki A, Wilks AF, Harpur AG, Sadowski HB, Gilman MZ and Darnell JE (1993). Polypeptide signalling to the nucleus through tyrosine phosphorylation of Jak and Stat proteins. *Nature* 366, 580-3.

Sprang SR and Bazan JF (1993). Cytokine structure taxonomy and mechanisms of receptor engagement. *Curr. Opin. Structural Biol.* 3, 815-827.

Takeda K, Tanaka T, Shi W, Matsumoto M, Minami M, Kashiwamura S, Nakanishi K, Yoshida N, Kishimoto T and Akira S (1996). Essential role of Stat6 in IL-4 signalling. *Nature* 380, 627-30.

Thierfelder WE, Vandeursen JM, Yamamoto K, Tripp RA, Sarawar SR, Carson RT, Sangster MY, Vignali DDA, Doherty PC, Grosveld GC and Ihle JN (1996). Requirement for STAT4 in interleukin-12- mediated responses of natural killer cells and T cells. *Nature* 382, 171-174.

Wakao H, Gouilleux F and Groner B (1994). Mammary gland factor (MGF) is a novel member of the cytokine regulated transcription factor gene family and confers the prolactin response [published erratum appears in *EMBO J* 1995 Feb 15;14(4):854-5]. *Embo Journal* 13, 2182-91.

Wen Z, Zhong Z and Darnell J Jr. (1995). Maximal activation of transcription by Stat1 and Stat3 requires both tyrosine and serine phosphorylation. *Cell* 82, 241-50.

Yi T, Mui AL, Krystal G and Ihle JN (1993). Hematopoietic cell phosphatase associates with the interleukin-3 (IL-3) receptor beta chain and down-regulates IL-3-induced tyrosine phosphorylation and mitogenesis. *Molecular & Cellular Biology* 13, 7577-86.

Yoshimura A, Ohkubo T, Kiguchi T, Jenkins NA, Gilbert DJ, Copeland NG, Hara T and Miyajima A (1995). A novel cytokine-inducible gene CIS encodes an SH2-containing protein that binds to tyrosine-phosphorylated interleukin 3 and erythropoietin receptors. *Embo Journal* 14, 2816-26.

- 42 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE WALTER AND ELIZA HALL INSTITUTE FOR MEDICAL RESEARCH

(ii) TITLE OF INVENTION: THERAPEUTIC AND DIAGNOSTIC AGENTS

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: DAVIES COLLISON CAVE

(B) STREET: 1 LITTLE COLLINS STREET

(C) CITY: MELBOURNE

(D) STATE: VICTORIA

(E) COUNTRY: AUSTRALIA

(F) ZIP: 3000

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: AU PROVISIONAL

(B) FILING DATE: 01-NOV-1996

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: HUGHES DR, E JOHN L

(C) REFERENCE/DOCKET NUMBER: EJH/EK

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: +61 3 9254 2777

(B) TELEFAX: +61 3 9254 2770

- 43 -

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CACGCCGCCC ACGTGAAGGC

20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTCGCCAATG ACAAGACGCT

20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1236 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..636

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

CGAGGCTCAA GCTCCGGGCG GATTCTGCGT GCCGCTCTCG CTCCTTGGGG TCTGTTGGCC -101
GGCCTGTGCC ACCCGGACGC CCGGCTCACT GCCTCTGTCT CCCCCATCAG CGCAGCCCCG -41
GACGCTATGG CCCACCCCTC CAGCTGGCCC CTCGAGTAGG -1

```

- 44 -

ATG GTA GCA CGC AAC CAG GTG GCA GCC GAC AAT GCG ATC TCC CCG GCA	48
Met Val Ala Arg Asn Gln Val Ala Ala Asp Asn Ala Ile Ser Pro Ala	
1 5 10 15	
GCA GAG CCC CGA CGG CGG TCA GAG CCC TCC TCG TCC TCG TCT TCG TCC	96
Ala Glu Pro Arg Arg Arg Ser Glu Pro Ser Ser Ser Ser Ser Ser Ser	
20 25 30	
TCG CCA GCG GCC CCC GTG CGT CCC CGG CCC TGC CCG GCG GTC CCA GCC	144
Ser Pro Ala Ala Pro Val Arg Pro Arg Pro Cys Pro Ala Val Pro Ala	
35 40 45	
CCA GCC CCT GGC GAC ACT CAC TTC CGC ACC TTC GCG TCC CAC TCC GAT	192
Pro Ala Pro Gly Asp Thr His Phe Arg Thr Phe Arg Ser His Ser Asp	
50 55 60	
TAC CGG CGC ATC ACG CGG ACC AGC GCG CTC CTG GAC GCC TGC GGC TTC	240
Tyr Arg Arg Ile Thr Arg Thr Ser Ala Leu Leu Asp Ala Cys Gly Phe	
65 70 75 80	
TAT TGG GGA CCC CTG AGC GTG CAC GGG GCG CAC GAG CGG CTG CGT GCC	288
Tyr Trp Gly Pro Leu Ser Val His Gly Ala His Glu Arg Leu Arg Ala	
85 90 95	
GAG CCC GTG GGC ACC TTC TTG GTG CGC GAC AGT CGT CAA CGG AAC TGC	336
Glu Pro Val Gly Thr Phe Leu Val Arg Asp Ser Arg Gln Arg Asn Cys	
100 105 110	
TTC TTC GCG CTC AGC GTG AAG ATG GCT TCG GGC CCC ACG AGC ATC CGC	384
Phe Phe Ala Leu Ser Val Lys Met Ala Ser Gly Pro Thr Ser Ile Arg	
115 120 125	
GTG CAC TTC CAG GCC GGC CGC TTC CAC TTG GAC GGC AGC CGC GAG ACC	432
Val His Phe Gln Ala Gly Arg Phe His Leu Asp Gly Ser Arg Glu Thr	
130 135 140	
TTC GAC TGC CTT TTC GAG CTG CTG GAG CAC TAC GTG GCG GCG CCG CGC	480
Phe Asp Cys Leu Phe Glu Leu Leu Glu His Tyr Val Ala Ala Pro Arg	
145 150 155 160	
CGC ATG TTG GGG GCC CCG CTG CGC CAG CGC CGC GTG CGG CCG CTG CAG	528
Arg Met Leu Gly Ala Pro Leu Arg Gln Arg Arg Val Arg Pro Leu Gln	
165 170 175	
GAG CTG TGT CGC CAG CGC ATC GTG GCC GCC GTG GGT CGC GAG AAC CTG	576
Glu Leu Cys Arg Gln Arg Ile Val Ala Ala Val Gly Arg Glu Asn Leu	
180 185 190	
GCG CGC ATC CCT CTT AAC CCG GTA CTC CGT GAC TAC CTG AGT TCC TTC	624
Ala Arg Ile Pro Leu Asn Pro Val Leu Arg Asp Tyr Leu Ser Ser Phe	
195 200 205	
CCC TTC CAG ATC TGA CCGGCTG CCGCTGTGCC GCAGCATTAA GTGGGGGCGC	676
Pro Phe Glu Ile * 3	
210	

- 45 -

```

CTTATTATTT CTTATTATTA ATTATTATTA TTTTCTGGA ACCACGTGGG AGCCCTCCCC      736
GCCTGGGTCTG GAGGGAGTGG TTGTGGAGGG TGAGATGCCT CCCACTTCTG GCTGGAGACC      796
TCATCCCACC TCTCAGGGGT GGGGGTGCTC CCCTCCTGGT GCTCCCTCCG GGTCCCCCCT      856
GGTTGTAGCA GCTTGTGTCT GGGGCCAGGA CCTGAATTCC ACTCCTACCT CTCCATGTTT      916
ACATATTCCC AGTATCTTTG CACAAACCAG GGGTCGGGGA GGGTCTCTGG CTCATTTTT      976
CTGCTGTGCA GAATATCCTA TTTTATATTT TTACAGCCAG TTTAGGTAAT AAACTTTATT     1036
ATGAAAGTTT TTTTTTAAAA GAAAAAAAAA AAAAAAAAAA     1075

```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 212 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Val Ala Arg Asn Gln Val Ala Ala Asp Asn Ala Ile Ser Pro Ala
 1             5             10             15
Ala Glu Pro Arg Arg Arg Ser Glu Pro Ser Ser Ser Ser Ser Ser Ser
      20             25             30
Ser Pro Ala Ala Pro Val Arg Pro Arg Pro Cys Pro Ala Val Pro Ala
      35             40             45
Pro Ala Pro Gly Asp Thr His Phe Arg Thr Phe Arg Ser His Ser Asp
      50             55             60
Tyr Arg Arg Ile Thr Arg Thr Ser Ala Leu Leu Asp Ala Cys Gly Phe
      65             70             75             80
Tyr Trp Gly Pro Leu Ser Val His Gly Ala His Glu Arg Leu Arg Ala
      85             90             95
Glu Pro Val Gly Thr Phe Leu Val Arg Asp Ser Arg Gln Arg Asn Cys
      100            105            110
Phe Phe Ala Leu Ser Val Lys Met Ala Ser Gly Pro Thr Ser Ile Arg
      115            120            125
Val His Phe Gln Ala Gly Arg Phe His Leu Asp Gly Ser Arg Glu Thr
      130            135            140

```

- 46 -

Phe	Asp	Cys	Leu	Phe	Glu	Leu	Leu	Glu	His	Tyr	Val	Ala	Ala	Pro	Arg
145					150					155					160
Arg	Met	Leu	Gly	Ala	Pro	Leu	Arg	Gln	Arg	Arg	Val	Arg	Pro	Leu	Gln
				165					170					175	
Glu	Leu	Cys	Arg	Gln	Arg	Ile	Val	Ala	Ala	Val	Gly	Arg	Glu	Asn	Leu
			180						185				190		
Ala	Arg	Ile	Pro	Leu	Asn	Pro	Val	Leu	Arg	Asp	Tyr	Leu	Ser	Ser	Phe
		195					200						205		
Pro	Phe	Gln	Ile												
	210														

DATED this 1st day of November, 1996

THE WALTER AND ELIZA HALL INSTITUTE
OF MEDICAL RESEARCH
By Its Patent Attorneys
DAVIES COLLISON CAVE

FIGURE 1

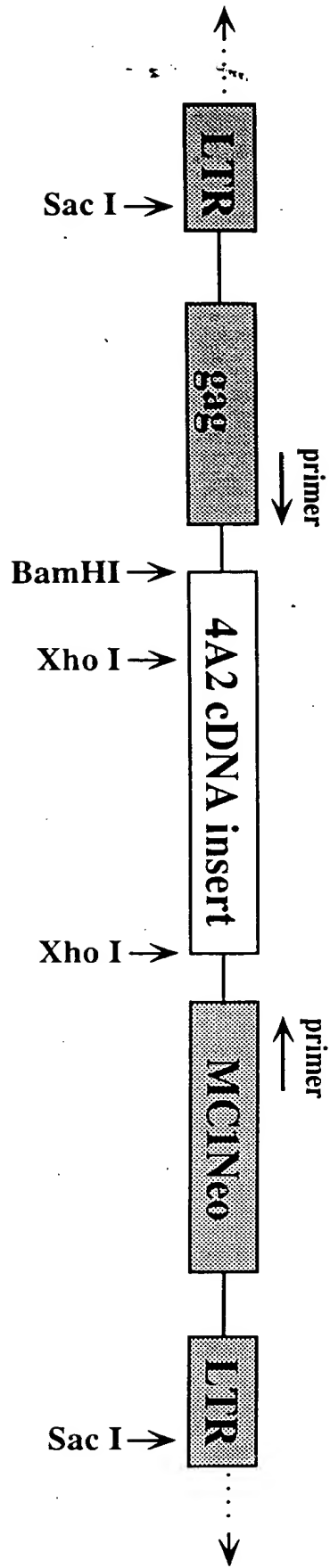
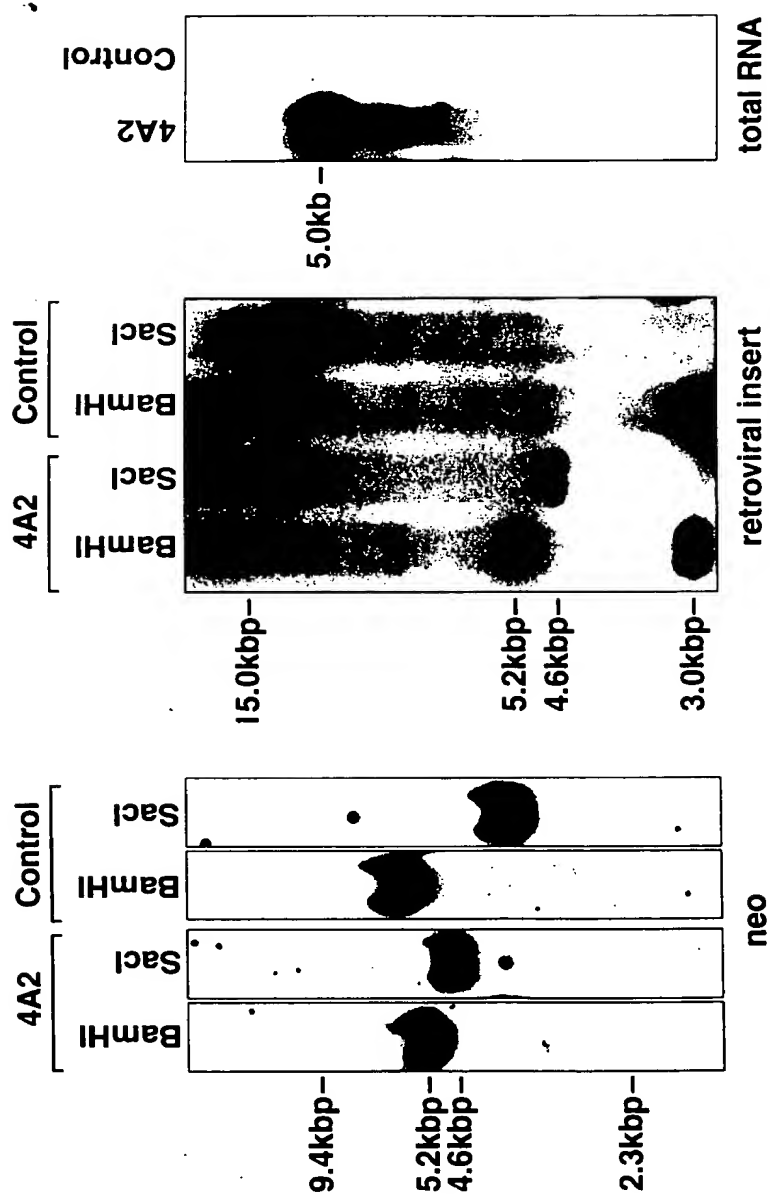
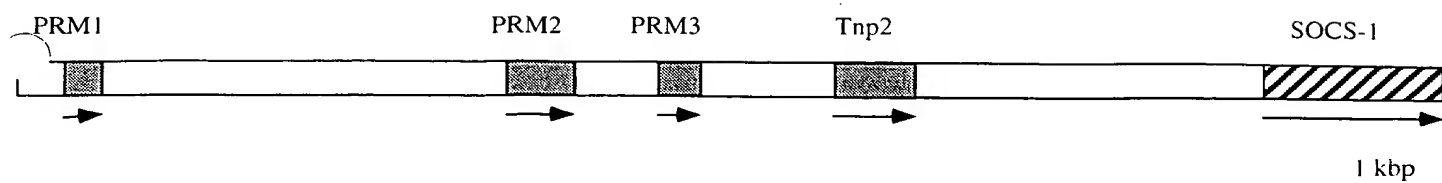


FIGURE 2



A



B

```

-159                      cgagggtcaagctccgggaggattctgcgtgccgctctcg
-120  ctcccttggggctctgttggccggcctgtgccacccggacgcccggctcaactgcctctgtct
-60   ccccatcagcgcagccccggacgctatggccacccctccagctggccctcgagtagg

1    M V A R N Q V A A D N A I S P A A E P R
1    ATGGTAGCACGCAACCAGGTGGCAGCCGACAATGCGATCTCCCCGGCAGCAGAGCCCCGA

21   R R S E P S S S S S S S S P A A P V R P
61   CGGCGGTCAGAGCCCTCCTCGTCTCGTCTTCGTCTCGCCAGCGGCCCCCGTGCGTCCC

41   R P C P A V P A P A P G D T H F R T F R
121  CGGCCCTGCCCCGGCGGTCCCAGCCCCAGCCCCTGGCGACACTCACTTCCGCACCTTCCGC

61   S H S D Y R R I T R T S A L L D A C G F
181  TCCCACTCCGATTACGGGCGCATCACGCGGACCAGCGCGCTCCTGGACGCCTGCGGCTTC

81   Y W G P L S V H G A H E R L R A E P V G
241  TATTGGGGACCCCTGAGCGTGCACGGGGCGCACGAGCGGCTGCGTGCCGAGCCCGTGGGG

101  T F L V R D S R Q R N C F F A L S V K M
301  ACCTTCTTGGTGCGCGACAGTCGTCAACGGAAGTCTTCTTCGCGCTCAGCGTGAAGATG

121  A S G P T S I R V H F Q A G R F H L D G
361  GCTTCGGGCCCCACGAGCATCCGCGTGAAGTCTTCCAGGCCGGCCGCTTCCACTTGGACGGC

141  S R E T F D C L F E L L E H Y V A A P R
421  AGCCGCGAGACCTTCGACTGCCTTTTCGAGCTGCTGGAGCAcTACGTGGCGGCGCCGCGC

161  R M L G A P L R Q R R V R P L Q E L C R
481  CGCATGTTGGGGGCCCCGCTGCGCCAGCGCCGCGTGGCGCCGCTGCAGGAGCTGTGTGCG

181  Q R I V A A V G R E N L A R I P L N P V
541  CAGCGCATCGTGGCCGCCGTGGGTGCGGAGAACCTGGCGCGCATCCcTCTTAACCCGGTA

201  L R D Y L S S F P F Q I *
601  CTCCGTGACTACCTGAGTTCCTTCCCCTTCCAGATCtgaccgggctgccgctgtgccgcag

661  cattaagtgggggagccttattatttcttattattaattattattatttttctggaacca
721  cgtgggagccctccccgcctgggtcggagggagtggttgtggaggggtgagatgcctccca
781  ctctctggctggagacctcatcccacctctcaggggtgggggtgctcccctcctggtgctc
841  cctccgggtccccctggtttagcagcttgtgtctgtgggcccaggacctgaattccactc
901  ctacctctccatgtttacatattcccagtatctttgcacaaaccaggggtcggggaggggt
961  ctctggcttcatttttctgctgtgcagaatcctatttttatatttttacagccagttta
1021 ggtaataaaaactttattatgaaagtttttttttaaaagaaaaaaaaaaaaaaaaaaaaa

```

FIGURE 3

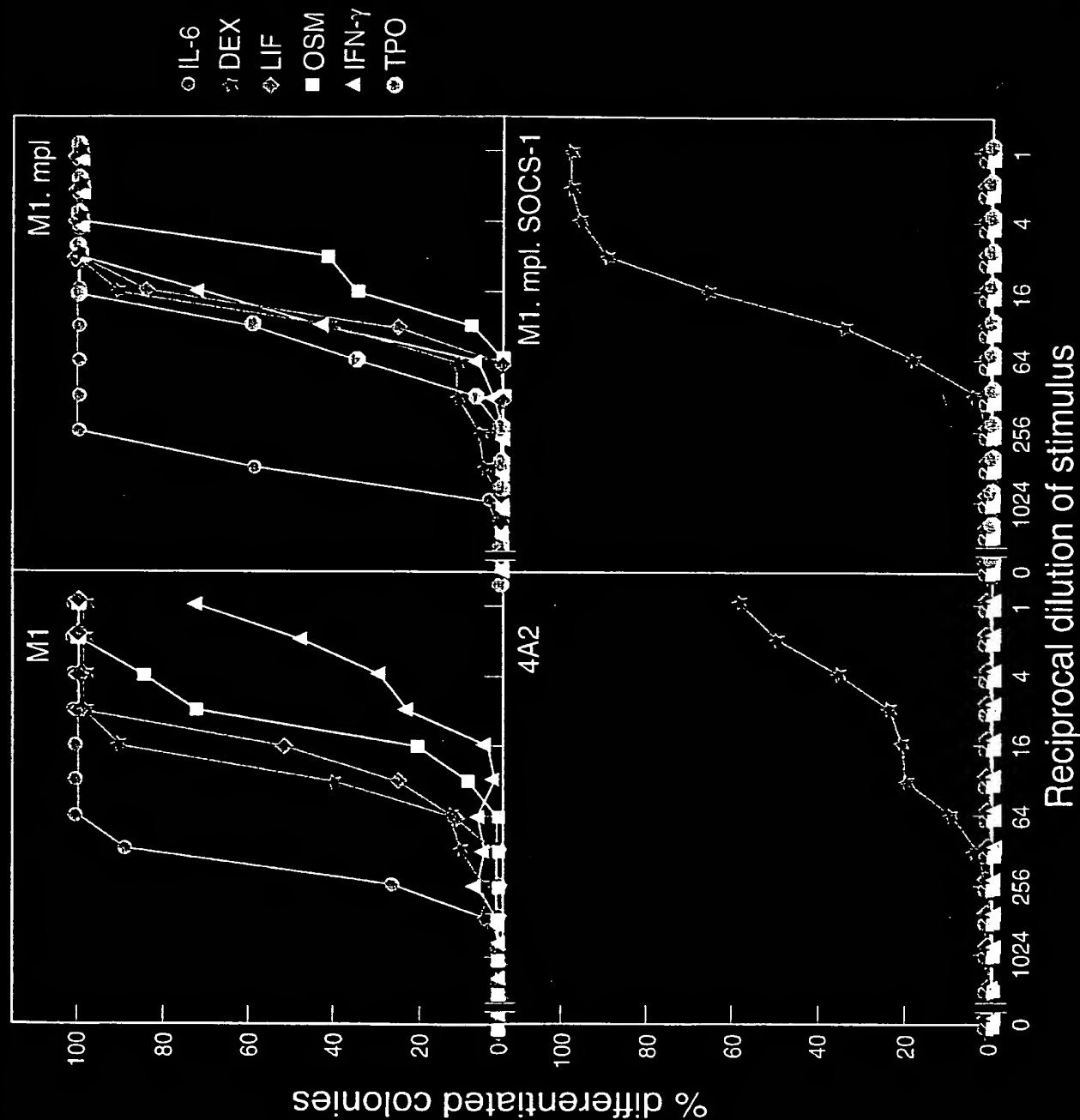


FIGURE 4

FIGURE 5

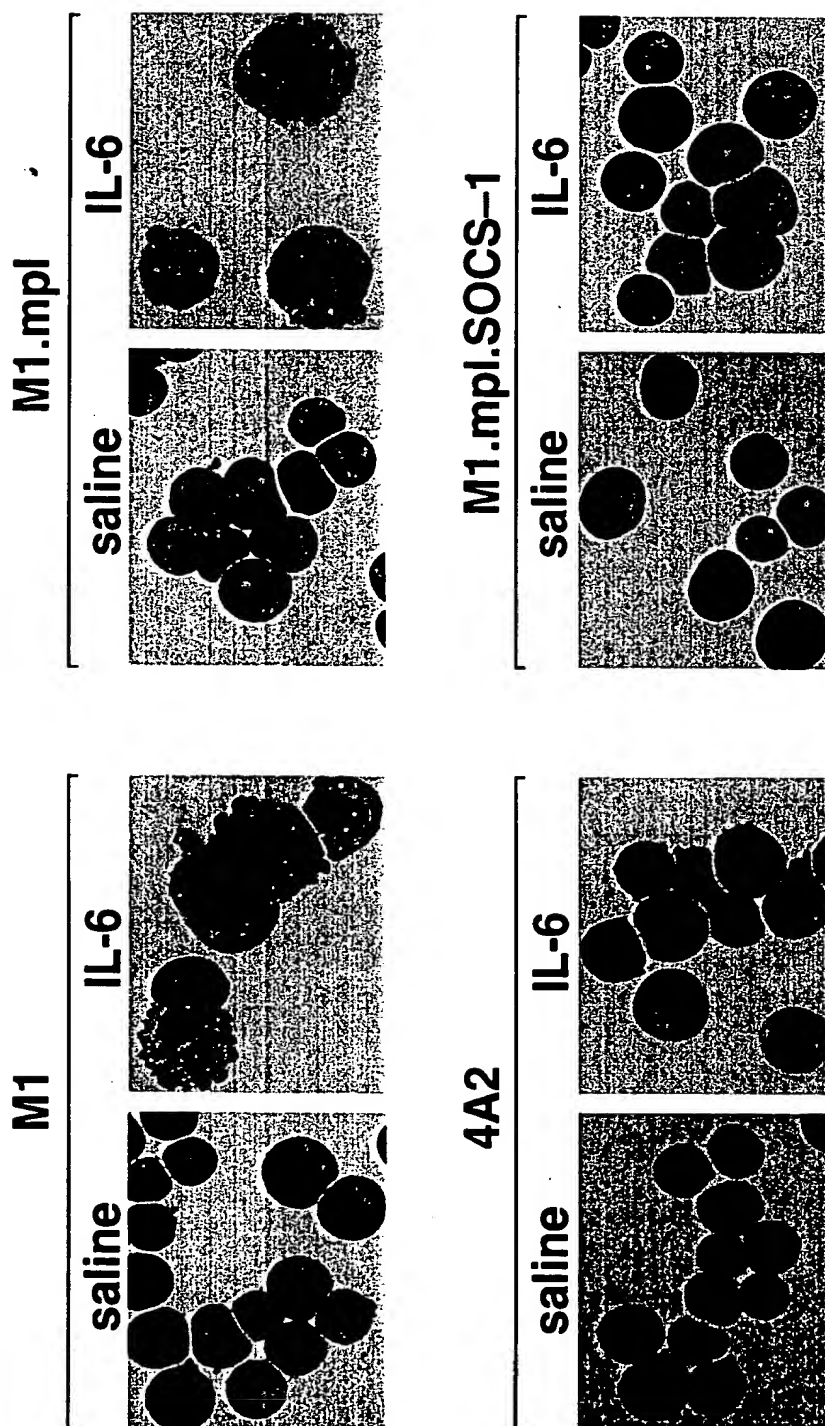
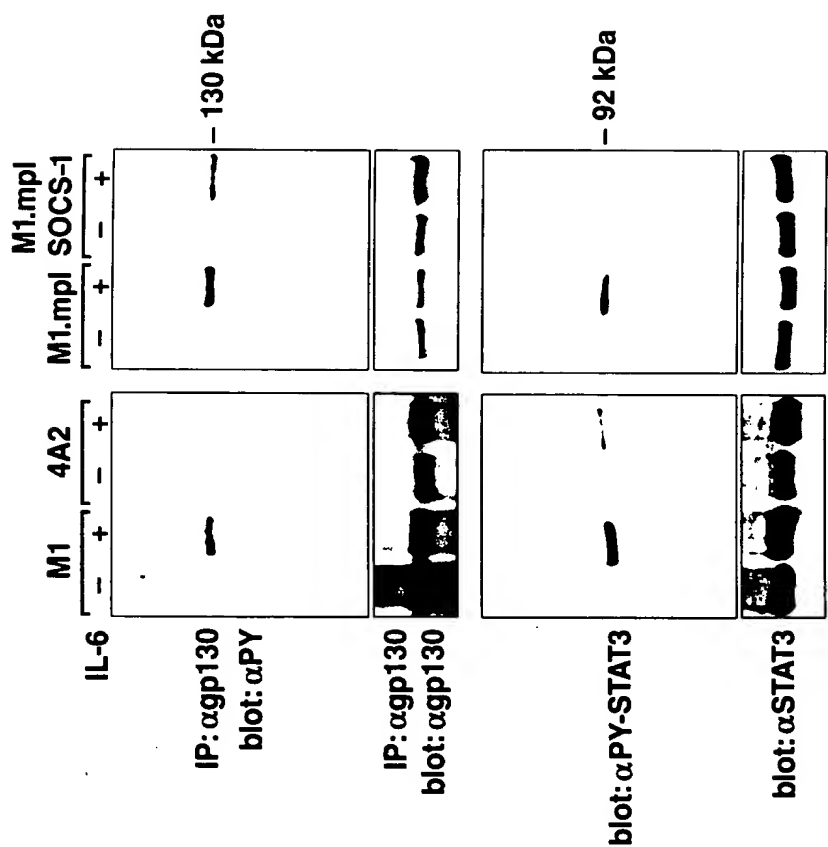


FIGURE 6



D:\DATA\PI\6A3STAR1.GEL 1996:10:03 11:00:52, Range = 0.01-266.25 Counts, 1.39

+	-	+	-	-	-	-	-	-	-	-	-	-
-	-	-	+	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	+	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	+	+	+	+	-

as 500
 as 500
 Cond. 100
 T. 10
 10-6



← SIF
 ← SIF
 ← SIF



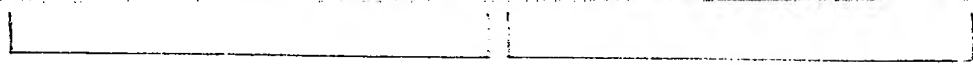
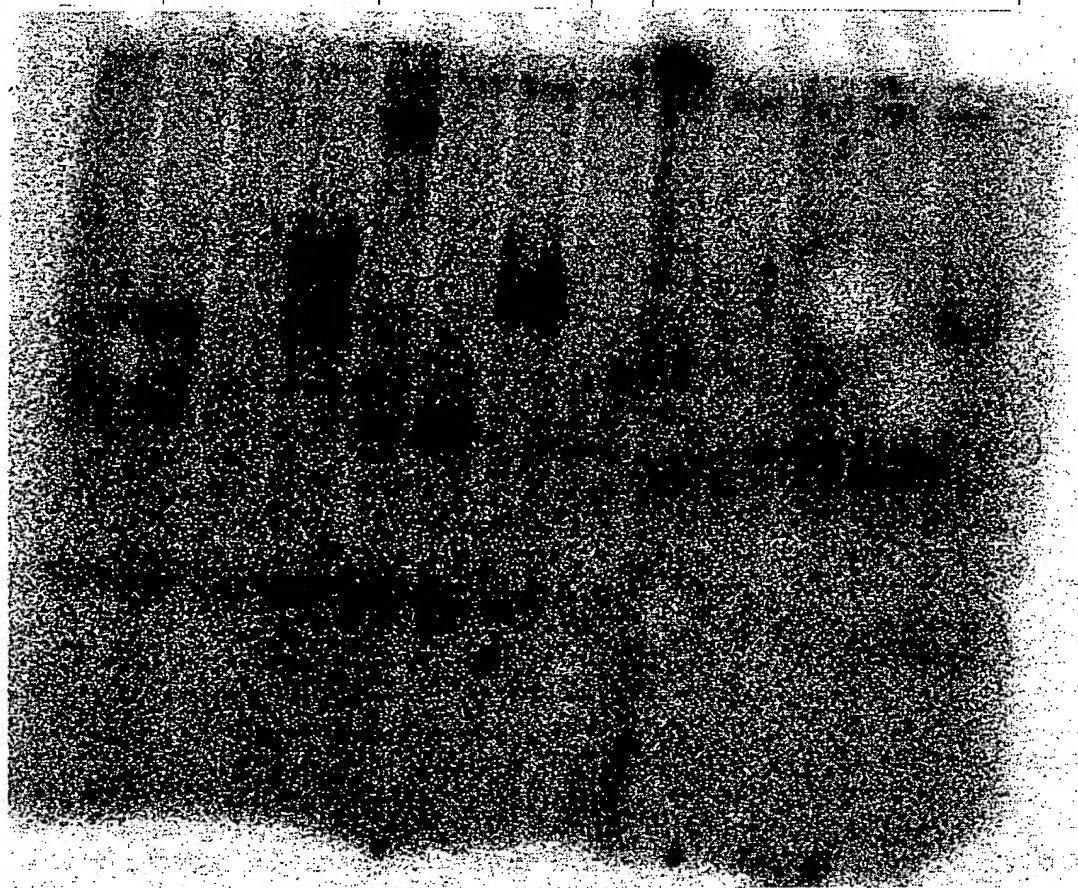
M1.mpl. 5005-1

M1.mpl

FIGURE 7A

2 STAR 2
 2 STAR 1
 1000 00
 1500 00
 2000 00

-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-
-	-	+	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	+	-	-	-	-	-
-	+	+	+	+	-	-	-	-	-	-	-



M1

4A2

FIGURE 7B

FIGURE 8

